Nitrogen setting Finish ophytic microbes: Herbaspirillum seropediceae Composed from Sugarcane parks

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ABSTRACT

Rahata taluka of Ahmednagar district is located between latitude 19.7127 and longitude 74.4833 meter.

Total 16 rhizospheric samples were collected from various localities of the taluka and soil testing was done to determine pH and Water Holding Capacity (WHC). After isolation of bacteria from soil sample, cell morphology and colony morphology was studied. With the help of special media and morphological characters preliminary identification of H. seropediceae was done.

seropediceae bacterial strains viz. Colonies were creamy white, circular, small to large sized, opaque with smooth margins. H. The study of H.

Key Words: Herbaspirillum seropediceae, Endophytic bacteria.

INTRODUCTION

The average temperature in Rahata taluka is 25.9°C and the average rainfall is 511 mm.

The area under Rahata is 1061.5 square kilometre (Mahadule et al., 2017). Gulve and Gadekar (2017) studied watershed development program in Ahmednagar district. Nitrogen fixation is an important process in plants providing Nitrogen as the most valuable macronutrient required by the plant. Crop rotation with legumes has been recognized to increase soil fertility and agricultural productivity (Cheng, 2008). Santi1 et al., (2013) studied b iological nitrogen fixation in non-legume plants. Endophytic bacteria can influence plant growth & productivity through Nitrogen fixation. Conceptually, plant growth promoting endophytic bacteria may affect plant growth either directly or indirectly (Sansanwal et al., 2017). Large and diverse populations of N2-fixing bacteria are associated with sugarcane.

Endophytic bacteria establish in between and within the spaces of all plant parts and not causing any plant disease. They create array of relationship include mutualism, cannibalistic, commensalistic and trophobiotic in nature. Endophytic bacteria play a major role in developing plant growth enhancement, phytoremediation, phosphate solubilization, nitrogen fixation, modulation of plant metabolism and phytohormone signaling.

The in plants (Alves et al., 2005). Endophytic bacteria are alternative to agrochemicals (fertilizers and pesticides) in developing environment friendly agriculture (Adeleke and Babolola, 2017).

Therefore, e ndophytic bacteria play an important role in microbial ecology, associating environmental factors, and their roles that contribute to their effectiveness in promoting plant growth for maximum agricultural crop productivity was highlighted . H. Its colonizing ability was evaluated in field of agriculture to promote the growth and development of crop plant. A preliminary study regarding contributions of the bacterial endophyte H.
H. seropediceae was found mainly inside cortical cells of stems and inside xylem vessels. No L-glucuronidase activity was observed in non-inoculated plants. H. seropediceae is able to increase nutrient supply, soil fertility and crop growth of sugarcane. The study of H. seropediceae will be useful for further researchers and it will be better alternative for chemical fertilizers. H. seropediceae colonizing the root intercellular spaces and the interior of root epidermal cells.

They proposed that H. seropediceae could be distributed from the base of the stem to other organs via stem xylem vessels, since they also detected xylem colonization in the basal region of the stalk in non-inoculated sugarcane plants (Baldani et al., 1992). Hence during the present investigation report of H. seropediceae was collected from sugarcane from Rahata taluka of Ahemednagar district

MATERIALS & METHODS

a) Collection of bacterial samples: Rhizosphere samples were collected from 16 different locations of Rahata taluka of Ahmednagar district in sterile zipped locked polythene bags. Those samples were brought to the laboratory and kept at 4OC for further investigations. Soil pH was calculated using pH meter, while Water Holding Capacity (WHC) was determined as described (Kalra, 1995).

b) Isolation of bacterial samples: One gram of soil was suspended in 10 ml distilled water to prepare soil suspension. It was inoculated on specific Herbaspirillum manitol agar media (Hi-Media) and incubated at 25±2OC for 48 Hrs.

c) Morphological Characterization: Confirmation of the bacteria was done by relevant morphological characterization (Phalke et al., 2017). Growth of colonies was observed after 48 Hrs. Morphology characterization of bacterial cell was studied in respect to cell size, shape and gram staining. While Colony morphology was studied in respect to color, shape, size, appearance and colony margins on the special culture media as described by Phalke et al., (2017). Cultures were preserved at 20OC for further studies.

RESULTS & DISCUSSION

The observations regarding soil type, soil pH and Water Holding Capacity (WHC) are presented in Table. Various types of soil were recorded in study area like Black soil, Regur soil and loamy soil. An average pH of soil samples collected from the study area was ranging between 5.7 to 6.5 pH. Maximum soil pH was recorded at Babhleshwar besides Pravara river north site (6.5); while minimum at tail tank side of Wakdi beside Khandoba Mandir north side (5.7 pH). Overall average pH of all samples collected from 20 localities was 6.1 Whereas 8 localities showed high pH than that of the average pH of all samples viz. Astgaon (6.1), Nighoj-Nimgaon (6.1), Rastapur (6.1), Aadgaon (6.2), Rampurwadi (6.2), Loni Kh.(6.3), Sadatpur (6.3), and Babhleshwar (6.5). While 12 localities showed less pH than that of the average pH viz. Chitali (6.0), Bhagwatipur (5.9), Ekrukhe (5.9), Ganeshnagar (5.9), Jalgaon (5.9), Rajuri (5.9), Savli-Vihir (5.9), Shingve (5.9), Sakuri (5.8), Walki (5.8) and Wakdi (5.7).

WHC in the study area was ranging between 35.60 to 41.80 %; maximum WHC was recorded in the sample collected from Loni Kh. (41.80%); while minimum at Astgaon (35.60%). The average WHC of all the samples is 38.56; out of which 11 soil samples showed high WHC than the average; while 9 samples showed less WHC than the average.

These strains are grouped as Group-I. While 05 bacterial strains RT05, RT07, RT12, RT16 and RT20 were different from one another and they are grouped in Group-II. Bacterial samples of Group-I were gram negative. While bacterial samples of Group-II were Gram positive in staining. Group-II cell size was larger than that of Group-I which was varying between 2.45 µm to 2.68 µm.

All the bacterial colonies of Group-I strains were white colored on the special media and in Group-II, Strain RT05, RH07, RH12, RT16, and RT20 showed creamy yellow color. The bacterial colonies of Group-I strains were circular in shape while Group-II showed irregular shape. Colony size of the Group-I was ranging between 1.25 mm to 1.39 mm while colony size of Group-II was ranging between 2.14 mm to 2.63 mm. Appearance of the Group-I bacterial strain is glistering while Group-II showed opaque colonies. The bacterial strain margins of Group-I showed entire margins while Group-II showed opaque margin. Group-I morphological characters resembled with H. seropediceae.

Biochemically 16 bacterial strains are grouped as Group-I viz. RT01, RT02, RT03, RT04, RT06, RT08, RT10,RT11, RT14, RT15 and RT18 showed positive (+) response for Starch Hydrolysis, Catalase, Urease (Urea Hydrolysis), Citrate, Indole production and Nitrate reduction. Only Gelatin hydrolysis biochemical test

showed negative (-) results. These Biochemical characters resembled with H. seropediceae. While RT09, RT13, RT17 and RT19 strains are grouped as Group-II, these showed variation in biochemical test such as RT09 sample showed Starch Hydrolysis, Catalase, Gelatine hydrolysis, Urease (Urea Hydrolysis), Citrate, Nitrate reduction biochemical test was positive (+) only Indole production test show negative (-) test, RT19 sample show Starch Hydrolysis, Catalase, Gelatine hydrolysis, Indole production, Citrate, Nitrate reduction biochemical test was positive (+) only Urease (Urea Hydrolysis) test show negative (-) test while RT13 and RT17 sample show Starch Hydrolysis, Catalase, Gelatine hydrolysis, Urease (Urea Hydrolysis) Indole production, , Nitrate reduction biochemical test was positive (+) only Citrate test show negative (-) test. Hence, this it was concluded that group I Showed similarity to H. seropediceae.

Varieties of sugarcane was described by various standard protocols such as Manual of directorate of sugarcane development, Government of India (Jan-2013), Manual of Improved /Hybrid Varieties of sugarcane. Abnave et al., 2017 was described average production of sugarcane in Maharashtra. Malavath, R.N., 2017 was described various soil types for sugarcane cultivation. Wakgari, T., 2017 was described physical and hydraulic properties of soil for sugarcane cultivation. Indian institute of sugarcane research, Lukhnow was described detail protocol of sugarcane production and management. Hase, C.P., 2017 was described sustainable sugarcane cultivation under monoculturing in Maharashtra. Kalra, Y.P., 1995 was described determination of soil pH by standard methods.

Similar bacterial cell and colony morphology of H. seropediceae was described by various research workers (Asis et al., 2000; Baldani et al., 1986 and Ureta et al., 1995). Pessae et al., (2017) reported colonization of sugarcane by H. seropediceae inhibited by high N-fertilization. Trovero et al., (2017) suggested improved methodology for isolation of H. seropediceae and confirmation of its endophytic habitat. Rosconi et al., in 2013 described role of nitrogen fixing family Acetobacteraceae in agriculture.

Similar characterization of Gluconacetobacter diazotrophicus is reported by Ahmed et al., (2016) isolated from sugarcane cultivated in Upper Egypt. Njoloma et al., (2006) also studied morphological characterization of Azotobacter spp. from various localities of Aurangabad district (MS). Oliveira et al., (2009) reported presence of H. seropediceae as nitrogen-fixing bacterium in sugarcane.

Sr. No.	Sample Code	Location	Site	Variety of Sugarcane	Physical Properties of Soil		
					Soil type	pH	WHC
1	RT01	Aadgaon	Pravara canol west side	CoM 0265	Black soil	6.2	41.20
2	RT02	Astgaon	Pravara college campus north side	CoM 0265	Black soil	6.1	35.60
3	RT03	Babhleshwar	Pravara river north side	CoM 0265	Regur soil	6.5	39.20
4	RT04	Bhagwatipur	West Side	Phule-MS 10001	Black soil	5.9	37.40
5	RT05	Chitali	Godavari river north side	CoM12085	Black soil	6.0	40.60

Sample collection

Sample code	Cell Morphology			Colony Morphology				
	Gram Staining	Cell Size (Avg.)	Cell Shape	Color	Shape	Size (Avg.)	Appearance	Margins
RT01	-ve	1.51 µm	Rod	Creamy White	Circular	1.25 mm	Glistering	Entire
RT02	-ve	1.62µm	Rod	Creamy White	Circular	1.32 mm	Glistering	Entire
RT03	-ve	1.78 µm	Rod	Creamy White	Circular	1.34 mm	Glistering	Entire
RT04	-ve	1.81 µm	Rod	Creamy White	Circular	1.27 mm	Glistering	Entire
RT05	+ve	2.56 µm	Large rod	Creamy yellow	Irregular	2.44 mm	Opaque	Rough
RT06	-ve	1.59 µm	Rod	Creamy White	Circular	1.39 mm	Glistering	Entire

Morphological characters of Nitrogen fixing Endophytic bacterial strains collected

Sample code	Starch Hydrolysis	Catalase	Urease (Urea Hydrolysis)	Citrate	Indole production	Nitrate Reduction	Gelatin Hydrolysis
RT01	+	+	+	+	+	+	-
RT02	+	+	+	+	+	+	-
RT03	+	+	+	+	+	+	-
RT04	+	+	+	+	+	+	-
RT06	+	+	+	+	+	+	-

Biochemical characters of Nitrogen fixing Endophytic bacterial strains collected from various locations

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